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Restoration Potential of *Ruppia maritima* and *Potamogeton perfoliatus* by Seed in the Mid-Chesapeake Bay

by Steve Ailstock and Deborah Shafer

DEFINITION: Reproductive potential is a measure of an organism's capacity to produce offspring. In plants, total reproductive potential is a combination of vegetative or clonal offspring and those resulting from seeds. However, because seeds are produced in quantity and are the propagules responsible for distribution of new ecotypes to new habitats, they are the most significant contributor to the reproductive potential of perennial species. Determining an organism's reproductive potential provides essential information for modeling of population change, establishment of protective management regimes, development of propagation protocols, and the evaluation of factors that limit reproductive success.

PURPOSE: This technical note outlines protocols for assessing the reproductive potential of *Ruppia maritima* and *Potamogeton perfoliatus,* two species of submerged aquatic plants that predominate in the mesohaline reaches of the mid-Chesapeake Bay. Once reproductive potential by seed is defined for healthy populations of these species, their life cycles can be evaluated to identify nondestructive methods of harvesting seeds for restoration projects. Such non-destructive methods would leave intact the roots and rhizomes that persist in the aquatic soils and that are associated with population maintenance through vegetative spread.

Data on reproductive potential would also allow managers to estimate the effects of herbivory. For example, in the Chesapeake Bay the non-native mute swan is a voracious feeder on submersed aquatic vegetation (SAV). Since expansion of SAV is a priority goal for all Bay restoration programs, a clear conflict exists between the presence of these swans and SAV restoration goals. Reproductive potential of the plants coupled with the consumptive data for swans would allow managers to calculate the swans' impact to plant reproduction.

BACKGROUND: Assessing species' reproductive potential provides a means of devising multiple strategies for producing plants in ways best suited to meeting a variety of planting objectives, such as restoration, experimentation, education, and mitigation. This information can be used to estimate the potential of natural populations to expand through recruitment and to identify pathways for population enhancement using aquaculture and restoration techniques. In this process, measurement of reproductive potential can be coupled to techniques for propagule harvesting, development of production methodologies, and implementation of management strategies that reduce impacts to reproduction of existing wild populations.

Assessment of plant reproductive potential, first successfully applied to *Zostera marina* (Orth 2003, Orth et al. 2000, Orth et al. 1994), has led to refinements in techniques that have steadily reduced the costs of plant production and establishment for this species. Methods are now in place for successful restoration of *Z. marina* on the scale of acres, at planting densities approximating natural

populations.[1] Such protocols are needed for many species common to the different habitats of the Chesapeake Bay estuary.

In the three decades since populations of submersed aquatic vegetation were first surveyed in the main stem of the Chesapeake Bay and its tidal tributaries, total acreage has increased from approximately 12,180 acres in 1971 to 85,252 acres in 2001 (Chesapeake Bay Program 2002). This increase is generally attributed to four species of underwater grasses, each capable of producing abundant seed. *Zostera marina* (eelgrass) and *R. maritima* (widgeon grass) dominate in the southern polyhaline portions of the Chesapeake, *R. maritima* and *P. perfoliatus* (redhead grass) in the mesohaline habitats characteristic of the mid-bay and tidal tributaries, and *P. perfoliatus* and *Vallisneria americana* (wild celery) in the oligohaline habitats of the northern Bay and freshwater tributaries. The role of seeds in the restoration and expansion of natural populations of *Z.marina* has been well-documented (Orth 2003; Harwell and Orth 1999, 2002a, 2002b; Orth et al. 1994, 2000; Fishman and Orth 1996; Moore et al. 1993). This work serves as a template for establishing reproductive potential and propagation strategies for other species of submersed aquatic grasses like *R. maritima* and *P. perfoliatus*.

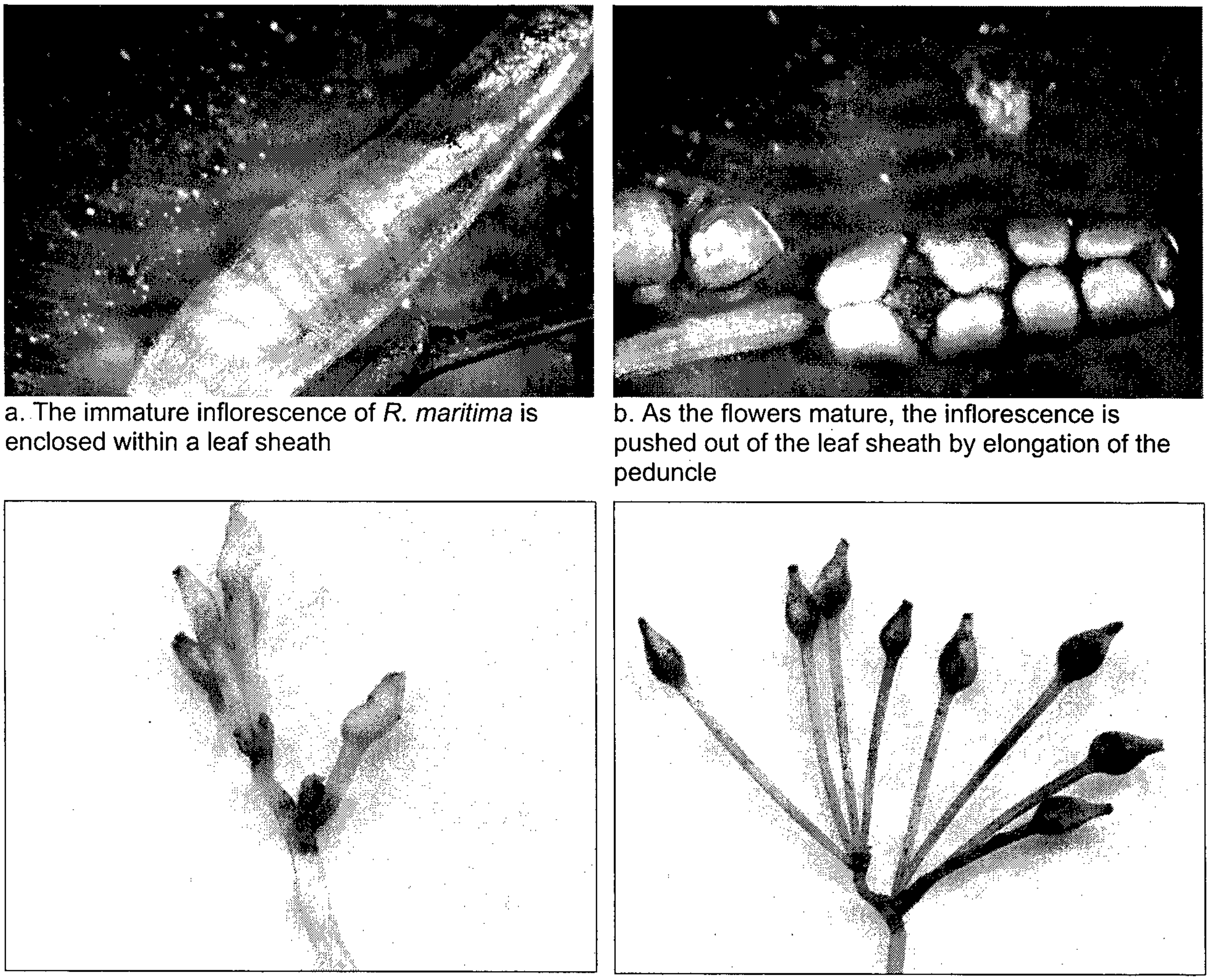
ASSESSMENT OF REPRODUCTIVE POTENTIAL

Ruppia maritima. *R. maritima* is a cosmopolitan submersed aquatic angiosperm common worldwide in brackish and saline waters. It persists through the formation of multibranched rhizomes located in the sediment and colonizes new niches through the formation of abundant seed. Inflorescences consist of two flowers, each having four carpels, which in turn contain a single ovule. Thus, a maximum of eight fruits, each containing a single seed, can be produced by each inflorescence in this species (Godfrey and Wooten 1979, Fernald 1970). During initial development, each immature inflorescence is enclosed within a leaf sheath, which is elevated in the water column at or near the surface by elongation of the main stems (Figure 1a). During anthesis, the individual mature flowers emerge from this sheath by elongation of the peduncle and pedicels until the eight individual fruits are fully formed (Figures 1b-1d).

Near the end of anthesis when many seeds are fully formed, the photosynthetic stems of *R. maritima* often weaken and break. These stems have near neutral density and float with their array of developing flowers and seeds to new locations according to prevailing wind and water currents. Under some conditions, the seed-laden stems accumulate along shorelines in dense piles of vegetation called wrack (Figure 2).

In July and August 2003, four collections of freshly accumulated *R. maritima* vegetative wrack were made from a healthy population in Slaughter Creek, a tributary of the Little Choptank River near Hooper Island, Maryland. Three 1/4-lb (fresh weight) samples from each collection were evaluated for the presence of floral structures, floral development, and presence of mature and immature seeds. Potential seed yields from these samples were calculated by multiplying the number of immature and mature inflorescences by eight, the number of seeds typically produced by an inflorescence, and then

[1] Personal communication, R. J. Orth, Professor of Marine Science, Virginia Institute of Marine Science, Gloucester Point, VA; and Dave Goshorn, Maryland Department of Natural Resources, Annapolis, MD.



a. The immature inflorescence of *R. maritima* is enclosed within a leaf sheath

b. As the flowers mature, the inflorescence is pushed out of the leaf sheath by elongation of the peduncle

c. After fertilization, growth of the pedicels exposes immature fruits

d. Mature fruits of *R. maritime* darken and eventually detach at the base of the pedicel

Figure 1. Anthesis of *R. maritima*

adding this number to the actual counts of immature and mature seeds in the 1/4-lb samples (Table 1). Counts from four samples of 25 stems taken in July and August 2003 yielded an average of 2.6 inflorescences per stem or 20.8 potential seeds per stem. Stem data were also used to confirm taxonomic descriptions of floral structures and to determine the actual number of inflorescences produced by stems. These data were normalized on a per-pound basis (fresh weight) to facilitate calculations of potential seed yield per collection. Mature seed yields were also normalized to units of seeds per pound to better compare yields from different collection dates and between species. These results are summarized in Table 1.

Figure 2. *R. maritima* wrack

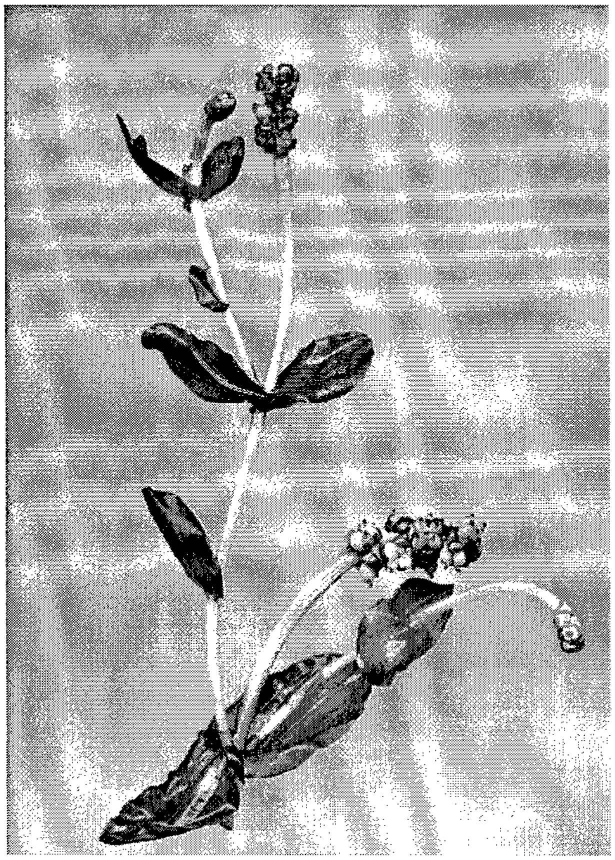
Table 1
Actual and Potential Seed Yields for Collections of *R. maritima* Vegetative Wrack

Collection Date	Pounds of Collected Wrack	Potential Seed Yield per Pound	Total Potential Seed Yield	Mature[1] Seed per Pound	Total Mature Seed Yield
07/28/03	475	2,648	1,257,800	347	164,825
08/01/03	450	2,277	1,024,650	431	193,950
08/05/03	781	1,194	932,514	217	169,477
08/20/03	250	83	20,750	60	15,000

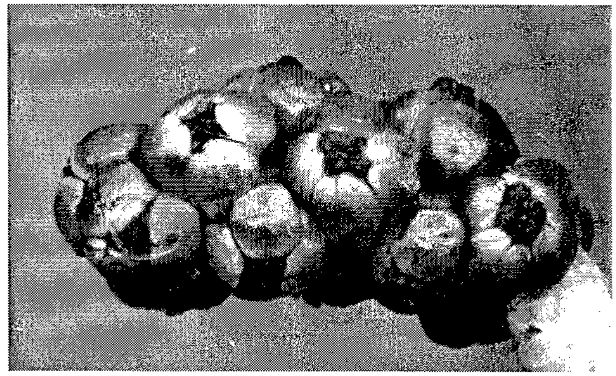
[1] Mature seed data are the result of actual counts of mature seed.

Potamogeton perfoliatus. *P. perfoliatus* is a broad-leaved submersed aquatic angiosperm found in calcareous ponds and brackish water habitats (Figure 3a). As a perennial, *P. perfoliatus* typically survives in winter by persistence of sparsely branched pale rhizomes embedded in the sediments. Inflorescences are variable, consisting of 5-12 flowers, (average 9), each consisting of 4 carpels which in turn contain a single ovule. Thus, seed formation ranges from 20-48 seeds per inflorescence. During early development, the terminal immature inflorescences are elevated to and slightly above the water surface by elongation of the peduncle (Figure 3b). During anthesis the individual flowers open in preparation for pollination and the subsequent developing seeds remain at or above the water surface during maturation and release (Figures 3c and 3d). This pattern of development places inflorescences near the surface where they can be harvested without damage to the underground structures by collecting only the top one third to one half of the vegetative stems.

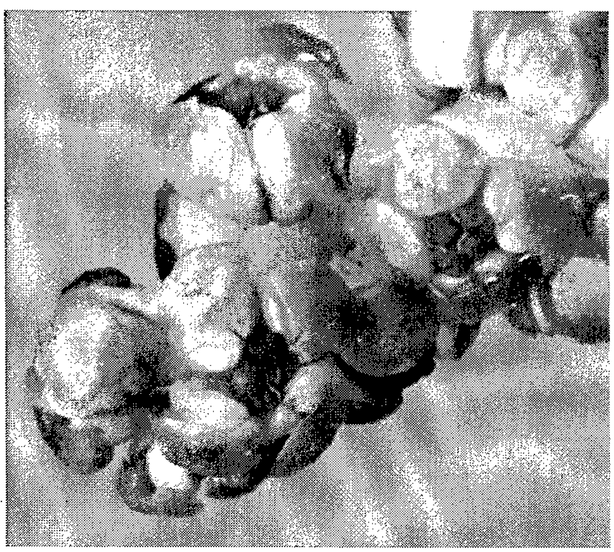
In July and August 2003, four collections of *P. perfoliatus* were taken in approximately 3 ft of water at MLW (mean low water) in Marshy Creek, a tributary of Fishing Bay near Kent Island, Maryland (Figures 4a and 4b). These collections were made by hand harvesting the upper one third to one half of the standing biomass, which was sufficient to harvest a majority (more than 90 percent) of the floral structures and seeds that were of a size that was readily visible. Counts of four samples of 25 stems each averaged 2.4 inflorescences per stem, producing a maximum seed yield that ranged from 48-115 seeds per stem as determined by the number of flowers making up the inflorescence. Three 1/4-lb samples from each collection were evaluated for the presence of floral structures, ranging from immature inflorescences to mature inflorescences bearing mature seed. Potential seed yields were calculated by multiplying the number of inflorescences by the average number of flowers/inflorescence (i.e., 36), times the usual number of seeds produced per flower (i.e., 4)) (Table 2). Mature seed yields were similarly determined using only the number of mature inflorescences having mature seed. All data were normalized on a per-pound (fresh weight) basis to facilitate comparison between sampling dates and species. These data are summarized in Table 2.



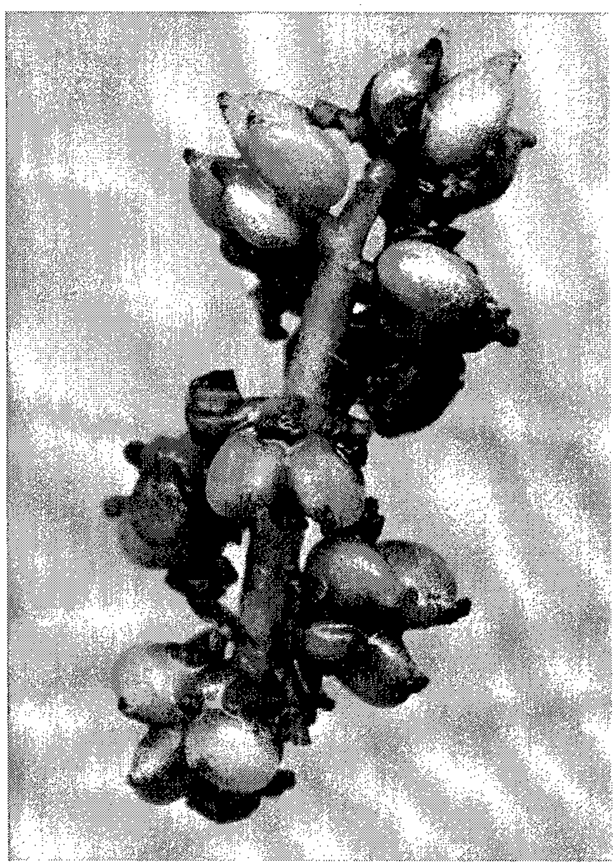
a. Photosynthetic stems of *P. perfoliatus* often produce multiple inflorescences that become elevated at or slightly above the surface of the water as they mature



b. Mature inflorescences are composed of 5-12 individual flowers

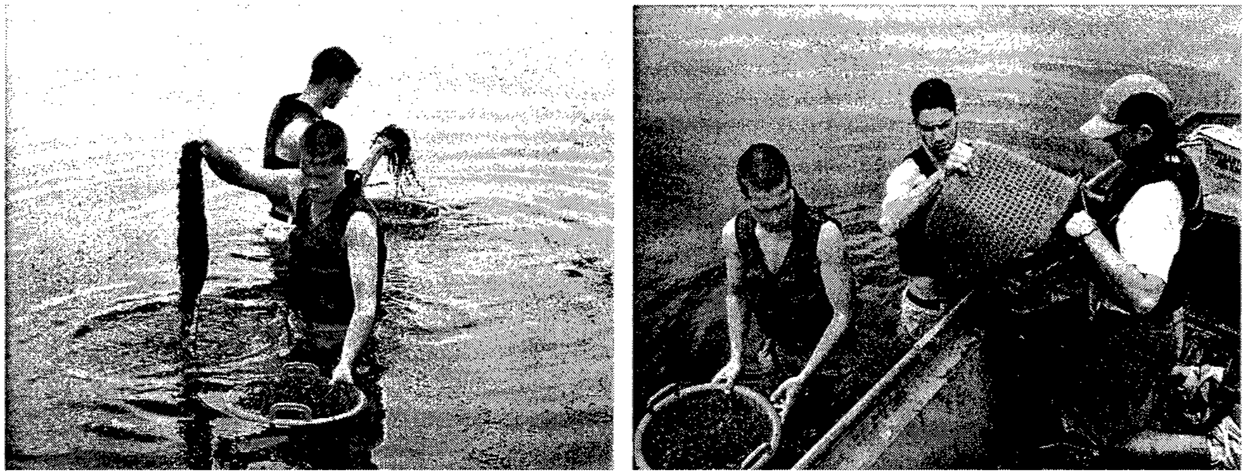


c. At maturity when the inflorescence reaches the water surface, the flower petals retract exposing the male and female reproductive structures



d. Clusters of four fruit pods at the end of the pedicle supporting each individual flower are evident prior to seed dispersal

Figure 3. Development of *P. perfoliatus*



a. *P. perfoliatus* flowers are restricted to the terminal portions of photosynthetic stems and can be harvested without damage to underground plant parts

b. After collection, seed bearing *P. perfoliatus* wrack is dewatered in preparation for transport and storage

Figure 4. Harvesting *P. perfoliatus*

Table 2
Actual and Potential Seed Yields for Collections of *P. perfoliatus* Vegetative Wrack

Collection Date	Pounds of Collected Wrack	Potential Seed Yield per Pound	Total Potential Seed Yield	Mature[1] Seed per Pound	Total Mature Seed Yield
07/29/03	275	17,374	4,777,850	7,439	2,045,725
08/07/03	286	22,270	6,369,220	10,415	2,978,690
08/14/03	273	15,024	4,101,552	11,088	3,027,024
08/27/03	210	11,134	2,338,140	3,839	806,190

[1] Mature seed yield was calculated by multiplying the number of inflorescences having mature seeds by 36.

Sources of Error. It is important to note that reproductive potential and seed yields are best determined over a number of years so that the influence of seasonal differences in growth can be minimized. Seasonal fluctuations in water quality, clarity, temperature, plant density, and factors such as localized herbivory can all influence reproductive success on an annual basis. Also, extrapolating seed yields from floral anatomy often assumes success rates for flower formation, floral abortion, pollination efficiency, fertilization, and maturation success of seeds that may not be found in nature. However, using actual counts of floral structures reduces this variability and the yields can be used to provide good estimates of reproductive potential for applications like the development of restoration strategies for a given species of underwater grasses or the evaluation of herbivores. With respect to restoration efforts, seeds of both *R. maritima* and *P. perfoliatus* are produced and can be harvested in large quantities using methods that are nondestructive to the parental stock. This same information can be used to estimate the impacts of herbivores (e.g. mute swans).

POTENTIAL FOR FURTHER USE: Many restoration efforts involving submerged aquatic vegetation (SAV) use whole plants collected from the wild, rather than seeds or commercially propagated plants. Major drawbacks of this approach include availability of suitable donor sites, concerns regarding impacts and recovery rates of donor sites following plant harvest, and limited genetic diversity of planting stock. Seeds are the preferred propagules for restoration programs requiring large numbers of inexpensive, genetically diverse stock. Their small size, uniform shape, and durability all contribute to a planting unit that is relatively easy to store, transport, and plant under field conditions common to submersed aquatic grasses.

Information on seed production of *R. maritima* and *P. perfoliatus* is applicable to collecting seeds, modeling propagule yields from existing populations, developing more cost-effective restoration techniques, and assessing the impacts of herbivores and can be applied to any other aquatic environments where these species are found. The process of customizing seed harvest techniques that are not destructive to the parental stock has application to all other species of perennial submersed aquatic angiosperms. The next step in the process of using seeds for restoration and expanding natural populations is to devise and test ways of isolating seeds from the harvested vegetative debris, storing the seeds and then planting the seeds in locations thought to be suitable for their subsequent growth and establishment.

IMPORTANT CONSIDERATIONS FOR PLANNING: Collecting seeds from wild populations of plants whose abundance is in question has become a feature of regulatory programs in many jurisdictions. Protection varies by regulatory status of the plant and the particular interests of federal and state government. In Maryland, notification and approval for collecting seeds from wild populations of underwater grasses is required by the Maryland Department of Natural Resources.[1] Efficient seed harvest for both *R. maritima* and *P. perfoliatus* requires healthy populations growing in protected areas, at high densities, where the plants are relatively free from major predation and environmental perturbations. Moreover, *R. maritima* donor beds must be specifically located in areas where prevailing winds and water currents move the detached seed bearing stems quickly on shore so that seeds are not lost in the water. These locations are particularly desirable because they maximize seed yields for the collector and the seeds collected are those that are unlikely to find suitable habitat for germination and growth. Seeds from *R. maritima* wrack are essentially lost from the natural pool of propagules and thus their use for restoration is particularly desirable. The cost of collecting seeds, while dependent on plant abundance and location, can be quite low and yields quite high. Additional information on planting protocols and methods of isolating, storing, and planting seeds must be gathered to optimize the biological return from these collections.

COSTS: Seed collections from natural populations are simple, low-technology endeavors whose yield and cost are dependent on the location, quantity, and reproductive success of the parental stock. The required materials can be as simple as boats, bags, and porous laundry baskets used for draining excess water. The collected material can be immediately moved to new locations and planted either by hand dispersal of the seeds or deployment in passive distribution devices similar to the buoys devised for *Zostera* seed distribution (Pickerell et al. 2003). However, if seeds are not immediately returned to the environment after collection, measures for storage are needed. Although

[1] Personal communication, Dave Goshorn, Maryland Department of Natural Resources, Annapolis, MD.

the specific storage requirements for maintaining viability of *R. maritima* and *P. perfoliatus* must be determined, regulation of temperature, moisture, and oxygen are thought to be important considerations. Providing these conditions will add to the expense of the projects and will be proportional to the volume of seeds to be processed.

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